

Reference: *Biol. Bull.* **203**: 260–261. (October 2002)

## Effects of Varying Salinity on Phytoplankton Growth in a Low-Salinity Coastal Pond Under Two Nutrient Conditions

Stacy Barron<sup>1</sup>, Carolyn Weber<sup>2</sup>, Roxanne Marino<sup>3</sup>, Eric Davidson<sup>4</sup>, Gabrielle Tomasky<sup>5</sup>, and Robert Howarth<sup>6</sup> (Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

Coastal ponds are highly susceptible to negative effects from nutrient loading (1). The usual approach for managing such systems is to reduce nutrient input. Another possibility for some low-salinity systems may be to control salinity if salinity has a pronounced influence on phytoplankton growth. Freshwater species generally compose the phytoplankton of low-salinity systems. One might expect growth to slow as salinity increases until the assemblage switches from freshwater to marine. Similarly, phytoplankton native to systems with fairly constant salinity through space and time may not tolerate any change in salinity, as they may be adapted to that specific salinity (Valiela, Boston University, pers. comm.).

Oyster Pond (Falmouth, MA) is a brackish pond connected to Vineyard Sound through a lagoon. The pond is currently mesotrophic to eutrophic (based on chlorophyll levels; 1), perhaps due to nutrient loading from the expanding residential population surrounding the pond. Oyster Pond's salinity has decreased from 32‰ (open to the ocean) to less than 2‰ (road restricting Vineyard Sound inflow) (2). Currently, dredging and a weir maintain the salinity at a fairly constant 2.3‰. Oyster Pond managers have the option of manipulating salinity within the pond *via* the weir. While managers plan to manipulate salinity according to which fish populations they desire in the pond (Barry Norris, Oyster Pond Environmental Trust), we are interested in considering what effects salinity changes might have on resident phytoplankton populations. To determine if the general Oyster Pond phytoplankton population could adapt to changes in salinity, we added excess nutrients (nitrate and phosphate) under three salinity regimes. To determine if cyanobacteria could adapt to changes in salinity under N-depleted conditions, we added excess phosphate.

Water was collected from the northern end of Oyster Pond. Three salinity treatments (0.2‰, 2.3‰, and 5.0‰) under two nutrient conditions were created by mixing sieved Oyster Pond water (150- $\mu$ m mesh to remove macrozooplankton), filtered Vineyard Sound water (GF/F), and deionized water in clear polycarbonate bottles. The 0.2‰ treatment contained 200 ml Oyster Pond water and 1800 ml deionized water. The 2.3‰ contained 200 ml Oyster Pond water, 129 ml Vineyard Sound water, and 1671 ml deionized water. The 5.0‰ treatment contained 200 ml Oyster Pond water, 298 ml Vineyard Sound water, and 1502 ml deionized

water. Three replicate bottles in each salinity treatment were enriched with  $\text{NaNO}_3$  and  $\text{NaH}_2\text{PO}_4$  to final concentrations of 50  $\mu\text{M}$  and 3  $\mu\text{M}$ , respectively (N + P), while another three bottles at each salinity were enriched only with  $\text{NaH}_2\text{PO}_4$  to a final concentration of 3  $\mu\text{M}$  (P). Ambient nitrate and SRP (surface reactive phosphate) concentrations in the pond were 0.2  $\mu\text{M}$  and less than 0.5  $\mu\text{M}$ , respectively. Since Vineyard Sound water used to set up the 2.3‰ and 5.0‰ salinity treatments contained some nitrate and SRP (0.01  $\mu\text{M}$  and less than 0.5  $\mu\text{M}$ , respectively), nutrient additions were in excess to avoid a systematic bias. Two mM  $\text{NaHCO}_3$  was added to each salinity treatment to buffer against  $\text{CO}_2$  depletion and pH changes (3). Bottles were incubated from 24–29 °C with a 15:9 light:dark cycle. Light intensity ranged from  $\sim 280$  to 350  $\mu\text{E m}^{-2}\text{s}^{-1}$ .

For the N + P enrichments, 100 ml of water was taken from each bottle initially and daily over 8 days. Chlorophyll *a* concentration was measured fluorometrically after overnight extraction in acetone (4). P additions were sampled similarly over 10 days; phytoplankton samples were preserved with Lugol's solution initially and at 10 days. Cyanobacterial heterocysts were estimated using an inverted microscope and Sedgwick-Rafter counting chamber.

Phytoplankton grew well at all three salinities in the N + P enrichment over time (Fig. 1). These data suggest that, given ample nutrients, phytoplankton from north Oyster Pond tolerate salinities ranging from 0.2‰ to 5.0‰; they do not appear to be closely adapted to ambient salinity. The short-term physiological response observed in this experiment suggests that controlling pond salinity in the 0.2‰ to 5.0‰ range is not likely to result in large differences in overall phytoplankton growth when both N and P are available at high levels. We note that salinity manipulations can have effects on higher trophic levels, which may affect phytoplankton production and are not addressed by these experiments.

In the P treatment, phytoplankton growth over time was significantly slower, characteristic of a cyanobacteria response, and lower than in the N + P addition. With P addition alone, growth was significantly greater at ambient salinity (2.3‰) than at 5.0‰ (Fig. 1). The 0.2‰ treatment had intermediate rates of growth that were not significantly different from other treatments (Fig. 1). These data indicate that phytoplankton growth under P-enriched and N-depleted conditions may be differentially affected by salinity. Cyanobacterial heterocysts increased during the experiment at all salinities, indicating that nitrogen fixation was probably occurring. The largest increase in heterocyst numbers was in the 2.3‰ treatment (1307  $\text{ml}^{-1}$  at 10 days vs. 6  $\text{ml}^{-1}$  initially), indicating that N-fixing cyanobacteria present in Oyster Pond seem best adapted to ambient salinity. The 0.2‰ and 5.0‰ treatments increased from 6  $\text{ml}^{-1}$  initially to 193 and 345  $\text{ml}^{-1}$ , respectively. Note that only one sample was counted for each treatment at 10 days, so the difference in heterocyst numbers at 0.2‰ and 5.0‰ is

<sup>1</sup> Bowdoin College, Brunswick, ME 04011.

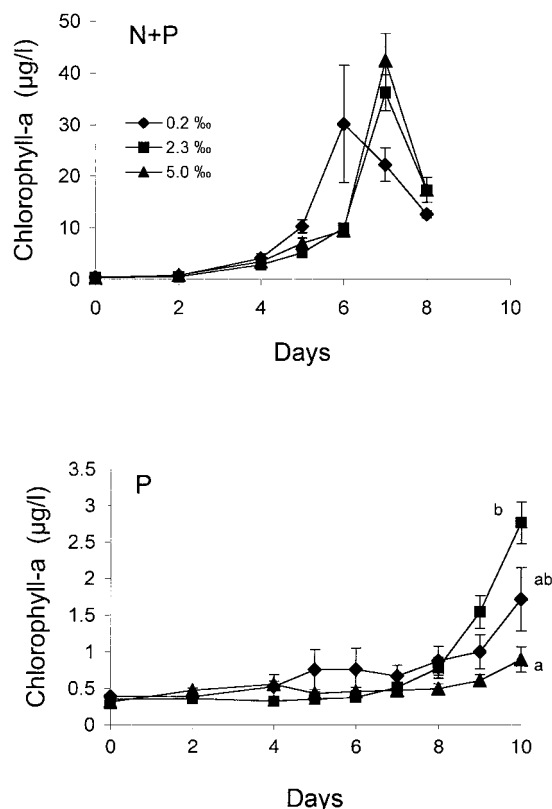
<sup>2</sup> Cornell College, Mount Vernon, IA.

<sup>3</sup> Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543.

<sup>4</sup> Woods Hole Research Center, Woods Hole, MA 02543.

<sup>5</sup> Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

<sup>6</sup> Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, and Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.



**Figure 1.** Chlorophyll *a* (mean  $\pm$  s.e.) through time in enrichment experiments done with N plus P additions and P additions only for three salinities (0.2‰, 2.3‰, and 5.0‰). Note scale differences on x and y axes. Different letters denote significant differences at the  $P < 0.05$  level using one-way ANOVA and Tukey's honest significant difference test. Water was collected from the northern end of Oyster Pond and mixed with deionized and Vineyard Sound water to produce the salinities. The experiment was done on July 21, 2002.

not statistically significant. The large increase in heterocysts in the 2.3‰ treatment may have influenced the final chlorophyll value by adding N to the water, allowing other species to grow.

The stimulation of phytoplankton growth in the P addition treatment contrasts with the finding of a companion study (5) which found that P additions to undiluted Oyster Pond water

incubated under the same conditions did not significantly increase phytoplankton biomass. Two differences may explain this. The experiment described here ran for twice as long, allowing more time for the typically slow-growing cyanobacteria, present in the pond water at very low abundances, to respond. Further, our P addition treatment had much lower inorganic N (owing to the 10-fold dilution of Oyster Pond water), which also may have provided conditions more favorable for heterocyst development and N fixation, resulting in enough increase in N availability to increase phytoplankton biomass. This apparent difference between the two experiments bears further experimental investigation.

This short-term experiment should be interpreted with caution because over time cyanobacteria might adapt to a change in salinity. Cyanobacteria can grow and fix N up to 32‰ salinity, although they do so more slowly at higher salinities (3). Also, heterocyst abundance in Oyster Pond is low compared to lakes with high rates of N-fixation (6). Thus N-fixing cyanobacteria may not be present in great enough numbers in Oyster Pond at this time of year to alleviate N-limitation. Nonetheless, these experiments suggest that there may be a potential in Oyster Pond for eutrophication in response to both P enrichment alone as well as to N + P enrichment. Thus, managers should consider the sources of and possible controls on both N and P inputs to the pond. Further, it does not appear that manipulating salinity within the range tested here (0.2‰–5‰) will substantially affect phytoplankton growth directly.

We thank Justin Minihane for help in the field and laboratory, the Ecosystems Center, BUMP, the Valiela lab, and OPET for the use of their facilities. This work was funded by a NSF Research Experience for Undergraduates grant (OCE-0097498).

### Literature Cited

1. National Research Council. 2000. *Managing Waste-water in Coastal Urban Areas*. National Academy, Washington, DC.
2. Emery, K. O. 1997. P. 111 in *A Coastal Pond Studied by Oceanographic Methods*, Oyster Pond Environmental Trust, Falmouth, Massachusetts.
3. Marino, R., F. Chan, R. Howarth, M. Pace, and G. E. Likens. 2002. *Ecosystems* (In press).
4. Clesceri, L., A. E. Greenberg, and A. Eaton. 1998. Pp. 10–20–10–21 in *Standard Methods for the Examination of Water and Waste-water*, 20th ed. United Book Press, Baltimore.
5. Weber, C. F., S. Barron, R. Marino, R. W. Howarth, G. Tomasky, and E. A. Davidson. 2002. *Biol. Bull.* 203: 261–263.
6. Howarth, R., T. Butler, K. Lunde, D. Swaney, and C. Ren Chu. 1993. *Limnol. Oceanogr.* 38: 1696–1711.

Reference: *Biol. Bull.* 203: 261–263. (October 2002)

### Nutrient Limitation of Phytoplankton Growth in Vineyard Sound and Oyster Pond, Falmouth, Massachusetts

Carolyn F. Weber (Cornell College, Mount Vernon, Iowa 52314), Stacy Barron<sup>1</sup>, Roxanne Marino<sup>2</sup>, Robert W. Howarth<sup>3</sup>, Gabrielle Tomasky<sup>4</sup>, and Eric A. Davidson<sup>5</sup>

<sup>1</sup> Bowdoin College, Brunswick, ME 04011.

<sup>2</sup> Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543.

<sup>3</sup> Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853.

<sup>4</sup> Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

<sup>5</sup> Woods Hole Research Center, Woods Hole, MA 02543.

Phytoplankton growth requires nitrogen (N) and phosphorus (P) in an approximate molar ratio of 16:1 (the Redfield ratio; 1). N or P limitation in an aquatic system is considered to occur when the availability of N relative to P is well below or above this ratio, respectively (2, 3). Past studies have shown that marine systems of moderate to high productivity are typically N limited, while sim-